Aqueous stable chitosan electrspun nanofibers for tissue engineering

Chaoxi Wu[1,2], Hengjie Su[2], Shunqing Tang[1], Warren O. Haggard[2], Joel D. Bumgardner[2]
[1]Department of Biomedical Engineering, Jinan University, [2]Department of Biomedical Engineering, University of Memphis

Statement of Purpose: There has been a growing interest in the fabrication of nanofibers derived from natural polymers due to their ability to mimic the structure and function of extracellular matrix. Electrospinning is a simple technique to obtain nano-micro fibers with customized fiber topology and composition. The chitosan electrospun nanofibers (CSEF) has recently been extensively studied due to the favorable properties of chitosan such as controllable biodegradation, good biocompatibility and high mechanical strength. Currently, chitosan can be electrospun from a solution of chitosan dissolved in either trifluoroacetic acid (TFA) or acetic acid (HAc)[1]. However, processes to remove residual acid and acid salts from the electrospun material generally result in a swelling of fibers and deterioration of the nano-fibrous structure. Crosslinking in combination with neutralization methods also have not been effective at preventing loss of nano-fibrous structure. Surface acylation of cellulose nanowhiskers has been reported to increase hydrophobicity but has not been attempted in chitosan nanofibers. Our hypothesis is that surface acylation renders CSEF insoluble in water and enables removal of residual acid from the electrospinning process while maintaining nano-fibrous structure and without causing cytotoxicity. This study aimed to evaluate acylation of CSEF and to characterize the aqueous stability and cytocompatiblity of the acylated CSEF.

Methods: Electrospinning: CSEF were prepared by electrospinning 5.5% wt/vol chitosan (71% DDA) in 70% TFA-30% methylene chloride solution at 25 kV onto a non-stick aluminum foil target rotating at 8.4 rpm. **Surface acylation:** CSEF were acylated in acetic anhydride with or without pyridine for 1 h at 20, 50 or 90 ^{o}C. After reaction, the membranes were washed in sodium carbonate and water to remove by-products and acid contaminants. **Characterization:** The acylated CSEF were characterized by immersion in PBS for stability, fourier transform infrared spectroscopy (FTIR) for chemical structure, and scanning electron microscope (SEM) for fiber morphology. Proliferation of osteoblast cells (SaOS-2) on membranes (n=5/membrane) was measured by the CellTitre Glow® (Promega) at 1, 3, and 5 days and compared to cast chitosan film controls. Cell morphology was observed by Live/Dead® stainning (Molecular Probes).

Results: Untreated CSEF swelled and dissolved in PBS. When CSEF membranes were acetylated without pyridine catalyst, only membranes at the 90 ^{o}C were stable in PBS, whereas in reactions with pyridine catalyst, even membranes acetylated at room temperature (20 ^{o}C) were stable in PBS. SEM evaluation (Fig. 1) showed that acylated CSEF exhibited nano-fibrous morphology after PBS immersion, but untreated CSEF membranes lost fibrous structure. FTIR spectrum of the acylated chitosan nanofibers showed a strong carbonyl absorption peak at 1751 cm-1, indicating successful acylation reactions. Fig. 2 showed that osteoblasts proliferated by 82 % on acylated CSEF over the 5 day culture and there was no difference in growth between the acylated CSEF or cast chitosan film controls. Osteoblast cells on acylated CSEF were viable and had low proportion of non-viable cells, as shown by live/dead staining (Fig.1c).

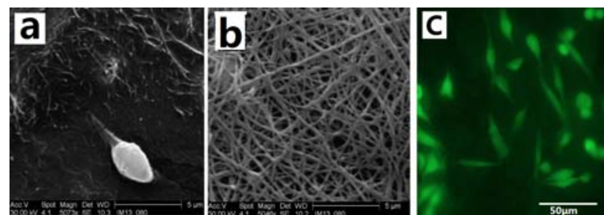

Figure 1. (a),(b) SEM image of the CSEF and acyl-CSEF after PBS immersion; (c) Live/dead stainning of SAOS-2 cells on acyl-CSEF.

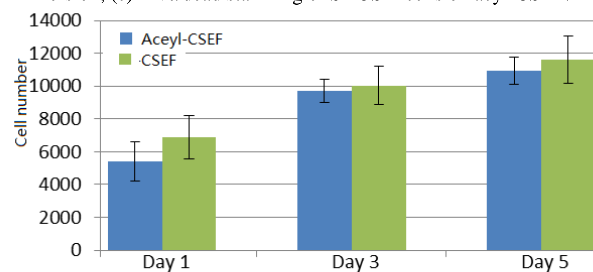

Figure 2.SAOS-2 cells proliferation over 5 days.

Discussion: Chitosan is an extensively studied natural polymer that has been used in tissue engineering and drug delivery applications. Electrospinning provide a new platform to fabricate fibrous biomaterials in a bottom-up approach. CSEF was first obtained by Ohkawa *et al* at 2004[2], but suffered from aqueous instability. Crosslinking by glutaraldehyde has been reported in 2007[3], but its toxicity raised a serious concern. $NaCO_3$ and NH_3OH neutralization were reported in 2006 and 2012[4,5], but the fibers deformed greatly after neutralization. This study presents a novel method without introducing harmful chemicals into the material to remove residual acids and retain desirable nanofibrous structure of CSEF. Acetyl moiety is chemically inactive and not likely to cause toxicity. The cell proliferation test found no toxicity of acylated chitosan nanofibers to SAOS-2 cells.

Conclusions: Our study identified surface acylation to be an effective strategy to stablize CSEF. The acylated CSEF were cytocompatible suggesting further investigation for tissue engineering.

References:

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